

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
27 May 2004 (27.05.2004)

PCT

(10) International Publication Number
WO 2004/043400 A2

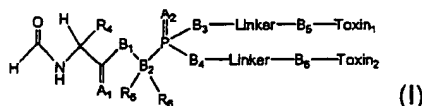
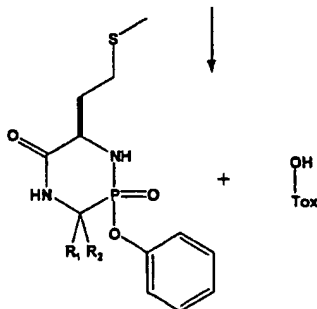
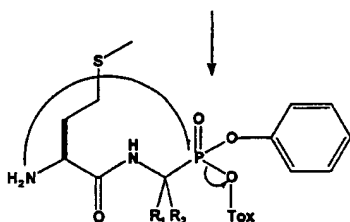
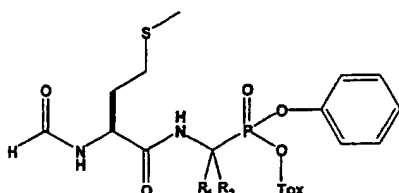
- (51) International Patent Classification⁷: A61K
(21) International Application Number: PCT/US2003/036124
(22) International Filing Date: 14 November 2003 (14.11.2003)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data: 60/426,771 14 November 2002 (14.11.2002) US
(71) Applicant (for all designated States except US): NEWBIOTICS, INC. [US/US]; 11760 Sorrento Valley Road, Suite E, San Diego, CA 92121-1018 (US).
(72) Inventors; and
(75) Inventors/Applicants (for US only): BALLATORE,

Carlo [IT/US]; 3990 Nobel Drive, Apt. #204, San Diego, CA 92122 (US). DOPPALAPUDI, Venkata, Ramana [IN/US]; 10986 West Ocean Air Drive, #278, San Diego, CA 92130 (US). SERGEEVA, Maria, V. [RU/US]; 11338 West San Raphael Driveway, San Diego, CA 92130 (US).

- (74) Agents: KONSKI, Antoinette, F. et al.; Bingham McCutchen LLP, Three Embarcadero Center, Suite 1800, San Francisco, CA 94111-4067 (US).
(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
(84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: PEPTIDE DEFORMYLASE ACTIVATED PRODRUGS



(57) Abstract: This invention provides compounds and methods for using them to inhibit the growth of microorganism that expresses Peptide Deformylase, the compounds having the general formula (I).

BEST AVAILABLE COPY

WO 2004/043400 A2



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- *without international search report and to be republished upon receipt of that report*

PEPTIDE DEFORMYLASE ACTIVATED PRODRUGS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/426,771, filed November 14, 2002, the contents of which hereby incorporated by reference into the present disclosure.

FIELD OF THE INVENTION

The present invention relates to the field of Enzyme Catalyzed Therapeutic Activation (ECTA™) therapy and in particular, ECTA therapies specific for microorganisms that express Peptide Deformylase ("PDF").

10

BACKGROUND

Throughout this disclosure, various publications are referenced by first author and date, within parentheses, patent number or publication number. The complete bibliographic reference is given at the end of the application. The disclosures of these references are hereby incorporated by reference into this disclosure to more fully describe the state of the art to which this application pertains.

Enzyme Catalyzed Therapeutic Activation (ECTA™) therapy is a novel technology that provides unique prodrug substrates for target enzymes. Unlike conventional therapies, ECTA prodrugs neither inhibit nor irreversibly inactivate the target enzyme. U.S. Patent Nos. 6,159,706; 6,245,750 and 6,339,151B1. See also PCT/US98/16607; PCT/US99/01332; and PCT/US00/20008.

Target enzymes convert the ECTA prodrug into a toxin preferentially within the target cell or in an environment wherein the target enzyme is expressed as compared to an environment where it is absent, as in an infected cell. Because the compounds do not require a targeting agent, they can be directly utilized, topically or systemically.

ECTA molecules do not, in most instances, yield cytotoxic products spontaneously (without target enzyme activation). They are not be appreciably activated by non-targeted enzymes, as this may result in toxicity to non-diseased or non-infected tissue. Table 1 summarizes the characteristics of ECTA molecules and

5 enzyme activators.

Table 1

| Characteristics of ECTA Target Enzymes | Characteristics of ECTA Prodrugs |
|---|---|
| Infectious Disease: Must be present only in target cells (including diseased cells, bacteria, fungi, etc.). The enzyme should be necessary for continued viability or pathogenicity. | Must be able to get into cells (by itself or as prodrug). |
| Must process a molecule that resembles the natural substrate (an ECTA molecule) into cytotoxic species. The resemblance only has to be significant with respect to the specificity of the enzyme/substrate interaction and the ability of the enzyme to process the substrate intracellularly into toxic species. | At least one of the products formed from the enzymatic reaction must be cytotoxic. However, the ECTA remain in inactive form until activated by the target enzyme. The compound must have a high degree of specificity for the targeted enzyme, although conversion by a non-targeted enzyme is acceptable if the product(s) are not cytotoxic. |
| Must not be inactivated by the ECTA molecule, intermediate(s), or the product(s) of the reaction. | Must not inhibit or deactivate the targeted enzyme. |

In cases of bacterial, viral and fungal infections in plants, people or agriculturally important animals, metabolic pathways being present in the pathogenic

10 organisms, but absent in the host are a source of potential ECTA target enzymes. For example, some pathways, as well as the enzymes involved, have only been found in bacteria, fungi and plants and not in mammalian cells. One example is the synthesis of "essential" amino acids - amino acids that animals cannot synthesize and must ingest with food. Nelson and Cox (1972).

Another example is Peptide Deformylase ("PDF", EC 3.5.1.88) which catalyses deformylation of N-terminal N-formyl methionine in a growing polypeptide chain. Meinnel (1999). The enzyme is present and active in bacteria (Meinnel et al, 1993), but has not been reported to be active in mammalian cells. Sequences
5 homologous to bacterial PDF sequences have been recently found in mammals but their exact function is unknown. Giglione (2000a) and (2000b).

Because the enzyme is not active in humans it has been used as a target for antibacterial drugs, mostly PDF inhibitors. Dithiols can act as non-specific PDF inhibitors by coordination of sulfhydryl groups with the active site metal ion.
10 Rajagopalan (1997). In case of 1,2- or 1,3- dithiols a slow extraction of the metal ion from the active site takes place. The formation of stable 5- or 6-membered rings, respectively, each containing two metal-sulfur bonds, accounts for this effect.

A rationally designed combinatorial library was used to select mechanism-based PDF inhibitors of the general structure HS-CH₂-CH(R_a)-CONH-CH(R_b)-
15 CONH-R_c. Wei et al. (2000a). The optimal inhibitor selected from the library possesses an n-Bu group as an R_a, R_b = -(CH₂)₃-NH-C(=NH)-NH₂, and R_c is 2-naphthalene. This compound acts as a competitive PDF inhibitor with a K_i of 15 nM.

Jayasekera et al. (2000) describes a series of non-peptidic compounds structurally related to the known anticholesteremic thyropropic acid to inhibit *E. coli*
20 PDF. Actinonin is reported to be a potent PDF inhibitor with activity in the subnanomolar K_i range. Chen (2000) and Guilloteau et al (2002).

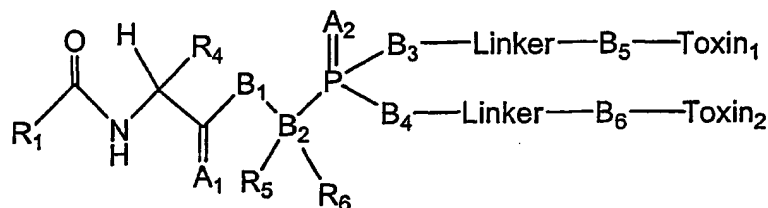
Wei, et al. (2000a) describe that 5'-dipeptidyl derivatives of 5-fluorodeoxyuridine release a small molecule (5-fluorodeoxyuridine (5-F-dUrd)) upon PDF catalyzed deformylation. 5-F-dUrd formation was monitored in the reaction of
25 the substrate catalyzed by purified PDF or *E. coli* crude lysates. The compound was marginally cytotoxic (IC₅₀ > 100 μM) when applied to *E. coli* bacteria. Potency was not increased by increased expression of PDF in bacteria (using a PDF-overexpressing strain). The compound was slightly more effective (IC₅₀ = 50 μM) against gram-positive microorganisms.

30 Additional inhibitors are described in Apfel et al. (2000), Apfel et al. (2001a), Apfel et al. (2001b), Clements et al. (2001), Durand et al. (1999), Chen et al. (2000), Yuan et al (2001), and Wise et al (2002)

However, a compound or agent that is not an inhibitor but rather selectively and effectively activated by PDF to a toxin has not been described. This invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

5 Thus, in one aspect, the present invention relates to a compound having the structure:



wherein:

R₁ is hydrogen or CH₃;

10 R₄ is selected from the group consisting of a substituted or unsubstituted aryl group, a substituted or unsubstituted, saturated or unsaturated C₁ – C₆ alkyl group and CH₂CH₂XCH₃, wherein X is selected from the group consisting of oxygen, sulfur and CH₂;

R₅ and R₆ are independently selected from the group consisting of hydrogen, a substituted or unsubstituted C₅ – C₁₄ aryl group and a substituted or unsubstituted, saturated or unsaturated C₁ – C₆ alkyl group;

20 =A₁ is either absent, in which case the carbon to which it was attached becomes a CH₂ group, or A₁ is selected from the group consisting of oxygen, sulfur, NH, NOH, NR₈, and CR₉R₁₀, wherein R₈, R₉ and R₁₀ are independently selected from the group consisting of hydrogen and C₁ – C₆ alkyl;

=A₂ is either absent, in which case the carbon to which it was attached becomes a CH₂ group, or A₂ is selected from the group consisting of oxygen, sulfur, NH, NOH and NR₁₁ wherein R₁₁ is selected from the group consisting of hydrogen and C₁ – C₆ alkyl;

25 B₁ is either absent or is selected from the group consisting of oxygen, sulfur, NR₁₂ and

CR₁₃R₁₄, wherein R₁₂, R₁₃ and R₁₄ are independently selected from the group consisting of hydrogen and a substituted or unsubstituted saturated or unsaturated alkyl;

B₂ is selected from the group consisting of carbon, oxygen and nitrogen,
 5 wherein, when B₂ is oxygen, R₅ and R₆ are absent and when B₂ is nitrogen, one of R₅ or R₆ is absent;

B₁ may be joined through R₁₂, R₁₃ or R₁₄ to R₅ or R₆ to form a saturated or unsaturated, substituted or unsubstituted ring which may contain 0 – 3 nitrogen atoms and/or 0 – 1 oxygen or sulfur atoms;

10 B₃ and B₄ are independently the same or different and are absent or are selected from the group consisting of oxygen, sulfur, NH, CH₂, NR₁₇, CHR₁₈, CR₂₁R₂₂ and, when either linker₁-B₅-Toxin₁ or linker₂-B₆-Toxin₂ is absent, OR₁₅, SR₁₆, NR₁₉R₂₀ and CR₂₃R₂₄R₂₅, wherein R₁₅ – R₂₅ are independently selected from the group consisting of hydrogen, substituted or unsubstituted, saturated or unsaturated
 15 alkyl and substituted or unsubstituted aryl;

B₃ or B₄ may be joined through one of R₁₅ - R₂₅ to B₁, R₅ or R₆ to form a saturated or unsaturated, substituted or unsubstituted ring which contains one phosphorus atom and which may contain 0 – 3 nitrogen atoms and/or 0 - 1 oxygen or sulfur atoms;

20 B₅ and B₆ are independently the same or different and are absent or are selected from the group consisting of oxygen, sulfur, OC(=O), SC(=S), OC(=O)NH, SC(=S)NH, OC(=S)NH, N(R₂₆) and C(R₂₇)(R₂₈), wherein R₂₆, R₂₇ and R₂₈ are independently selected from the group consisting of hydrogen and a substituted or unsubstituted, saturated or unsaturated alkyl;

25 Linker₁ and Linker₂ are independently the same or different and are absent or present and, if present, are traceless; and,

one of Toxin₁ or Toxin₂ may be absent.

In an aspect of this invention, R₁ is hydrogen and R₄ is CH₂CH₂XCH₃, and X is selected from the group consisting of CH₂, sulfur and oxygen.

30 In an aspect of this invention, X is sulfur.

In an aspect of this invention, A₁ and A₂ are both oxygen.

In an aspect of this invention, Linker₁-B₅-Toxin₁ is absent.

In an aspect of this invention, B₁ is absent.

In an aspect of this invention B₂ is carbon.

In an aspect of this invention, B₂ is nitrogen.

In an aspect of this invention, B₃ is selected from the group consisting of OH, OCH₃, OCH₂CH₃ and OC₆H₅.

5 In an aspect of this invention, Linker₂ is selected from the group consisting of substituted or unsubstituted allyl; substituted or unsubstituted benzyl;

C₆H₄CH₂X₁C(=X₂), wherein X₁ and X₂ are independently selected from the group consisting of oxygen, sulfur and N(R₂₉), wherein R₂₉ is hydrogen or C₁ – C₆

10 alkyl; and,

(CH₂)_nN(R)C(=O), wherein n is 2 or 3 and R is hydrogen or C₁ – C₆ alkyl.

In an aspect of this invention B₆ is absent.

In an aspect of this invention, in any of the above compounds, Toxin₁ and Toxin₂ are independently selected from the group consisting of aminoglycosides, mitomycin, CC-1065, ducarmycin, cyclopropyl indole, cyclopropyl benzoindole
 15 analogs, anthracyclins, vinca alkaloids, mitomycins, bleomycins, penicillins, cephalosporins, oxacillins, carbopenems, tetracyclins, chloramphenicols, macrolides, cycloserines, fluoroquinolones (including, but not limited to, ciprofloxacin and norfloxacin), glycopeptides, aminoglycosides, peptide antibiotics, oxazolidinones, quinolones, sulfonamides, cytotoxic nucleosides, pteridine family, nitrogen mustards,
 20 polyhalogenated biaryl ethers, diynes, podophillotoxins, taxoids, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloromethotrexate, mitomycin C, porfiromycin, 6-mercaptopurine, cytosine arabinoside, podophillotoxin, etoposide, etoposide phosphate, melphalan, vindesine, vinblastine, vincristine, leurosine, leurosine, bis-(2-chloroethyl)amine,
 25 trichlorcarban, trichlorocarbanilide, triclosan, tribromosalicylanilide, sulphamethoxazole, chloramphenicol, cycloserine, trimethoprim, chlorhexidine, hexachlorophene, fentichlor, 5-chloro-2-(2,4-dichlorophenoxy)phenol, 4-chloro-2-(2,4-dichlorophenoxy)phenol, 3-chloro-2-(2,4-dichlorophenoxy)phenol, 6-chloro-2-(2,4-dichlorophenoxy)phenol, 5-chloro-2-(3,4-dichlorophenoxy)phenol, 5-chloro-2-(2,5-dichlorophenoxy)phenol, 5-chloro-2-(3,5-dichlorophenoxy)phenol, 2,2'-dihydroxy biphenyl ether, halogenated 2-hydroxybenzophenones, 2-mercaptopyridine-N-oxide, combretastatin, camptothecin, apoptolidene, cisplatin,

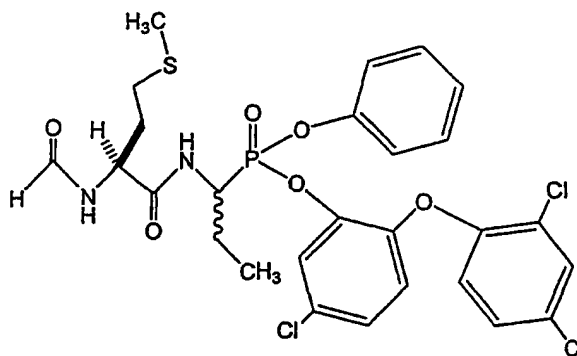
epothilone, halichondrin, hemiasterlin, methioprim, thapsigargin, chloroquine, 4-hydroxycyclophosphamide, etoposide, colchicine, melphalan, quercetin, genistein, erbstatin, N-(4-aminobutyl)-5-chloro-2-naphtalen-sulfonamide, pyridinyloxazol-2-one, isoquinolyloxazolone-2-one, verapamil, quinine, quinidine, and chloroquine.

5 In an aspect of this invention, Toxin₂ is a quinolone.

In an aspect of this invention, Toxin₂ is selected from the group consisting of triclosan, cyclopropylindole, cyclopropylbenzoindole and derivatives thereof.

In an aspect of this invention, B₁ is joined to R₅ or R₆ to form a ring.

An aspect of this invention is a compound having the structure:



10

An aspect of this invention is a composition comprising a compound hereof and a pharmaceutically acceptable carrier.

15 An aspect of this invention is a method for the treatment of a disease caused by a microorganism expressing a peptide deformylase enzyme, comprising administering an effective amount of a compound of claim 1 to a subject in need thereof.

Also provided by this invention is a method for inhibiting the growth of a microorganism that expresses PDF by contacting the microorganism with an effective amount of the compound as describe above. This method inhibits the growth of a gram-positive or gram-negative microorganism, *e.g.*, of *S. aureus*, *S. epidermidis*, *K. pneumoniae*, *E. aerogenes*, *E. cloacae*, *M. catarrhalis*, *E. coli*, *E. faecalis*, *H. influenzae* or *P. aeruginosa*. This method can be practiced *in vitro*, *ex vivo* and *in vivo*. Further provided is a method for alleviating the symptoms of an infection by a PDF expressing microorganism in a subject by administering or delivering to the subject an effective amount of the compound described above. A "subject" is defined herein and includes mammals such as human patients.

20

25

This invention also provides a composition comprising the prodrug compounds as described above, alone or in combination with other compounds or other agents, known or yet to be discovered, and a carrier. In one aspect, the carrier is another molecule or an inert substance such as a plate or column. In an alternative
5 embodiment, the carrier is a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are known in the art and described briefly above.

DETAILED DESCRIPTION OF THE INVENTION

Brief description of the table

Table 1 shows a list of non-limiting characteristics of ECTA target enzymes
10 and ECTA prodrugs.

Table 2 is a non-limiting list of bacteria that express PDF and therefore are expected to be susceptible to the compounds of this invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a proposed reaction scheme for PDF activation of the compounds
15 of this invention.

Discussion

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology,
20 which are within the skill of the art. Such techniques are explained fully in the literature, such as, "MOLECULAR CLONING: A LABORATORY MANUAL" Second Edition (Sambrook et al., 1989); "OLIGONUCLEOTIDE SYNTHESIS" (M.J. Gait, ed., 1984); "ANIMAL CELL CULTURE" (R.L. Freshney, ed., 1987); the series "METHODS IN ENZYMOLOGY" (Academic Press, Inc.); "HANDBOOK OF EXPERIMENTAL IMMUNOLOGY"
25 (D.M. Weir & C.C. Blackwell, eds.); "GENE TRANSFER VECTORS FOR MAMMALIAN CELLS" (J.M. Miller & M.P. Calos, eds., 1987); "CURRENT PROTOCOLS IN MOLECULAR BIOLOGY" (F.M. Ausubel et al., eds., 1987, and periodic updates); "PCR: THE POLYMERASE CHAIN REACTION" (Mullis et al., eds., 1994); "CURRENT PROTOCOLS IN IMMUNOLOGY" (J.E. Coligan et al., eds., 1991); J. March, ADVANCED ORGANIC

CHEMISTRY: REACTIONS, MECHANISMS AND STRUCTURE, 4th edition (John Wiley & Sons, NY (1992)).

As used herein, certain terms may have the following defined meanings.

The singular form "a," "an" and "the" include plural references unless the
5 context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude others. "Consisting essentially of,"
when used to define compositions and methods, shall mean excluding other elements
10 of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method
15 steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

As used herein, the term "peptidomimetic" refers to a compound containing non-peptidic structural elements that is capable of mimicking or antagonizing the biological action(s) of a natural parent peptide.

20 As used herein, the term "substituents" includes, but is not limited to halogen atoms and halomethyl groups such as CF₃ and CCl₃; oxygen containing groups such as oxo, hydroxy, carboxy, carboxyalkyl, alkoxy, alkoyl, alkoyloxy, aryloxy, aryloyl, and aryloyloxy; nitrogen containing groups such as amino, alkylamino, dialkylamino, cyano, azide and nitro; sulfur containing groups such as thiol, alkylthiol, sulfonyl and
25 sulfoxide; heterocyclic groups which may themselves be substituted; alkyl groups which may themselves be substituted; and aryl groups which may themselves be substituted.

The term "alkyl" refers to any branched or unbranched, cyclic or acyclic, saturated (alkyl) or unsaturated (alkenyl or alkynyl) hydrocarbyl radical. This term is
30 not limited to a monovalent radical. Where cyclic, the alkyl group is C₃ to C₁₂. Where acyclic, the alkyl group is C₁ to C₁₆. The term "alkoxy" and "aryloxy" means alkyl-O and aryl-O groups, respectively. Reference to "alkoyl" and "aryloyl" groups means alkyl-CO and aryl-CO, respectively.

The term "aryl" refers to an aromatic group, such as phenyl or naphthyl, or a heteroatomic group containing one or more heteroatom, such as pyridyl, pyrrolyl, furanyl and thiophenyl. This term is not limited to a monovalent radical. The term "aromatic" refers to any compound characterized by the presence of at least one
5 benzene ring. Examples of aromatic groups include, but are not limited to, benzene and naphthalene.

The alkyl and aryl groups may be substituted or unsubstituted. The term "aliphatic" refers to any and all organic compounds of hydrogen and carbon characterized by a straight chain of the carbon atoms. The subgroups of "aliphatic"
10 compounds includes alkanes, alkenes, and alkynes. The aliphatic groups may be unsubstituted or substituted. See above for examples of substituents.

The term "alicyclic" refers to and covers any and all organic compounds of hydrogen and carbon atoms joined to form one or more rings. The alicyclic groups may be unsubstituted or substituted. See above for examples of substituents.
15 "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, or cyclopentyl. This term is not limited to a monovalent radical. The cycloalkyl groups may be unsubstituted or substituted. See above for examples of substituents.

The term "alkenyl" refers to normal alkenyl, branch chain alkenyl and cycloalkenyl groups having one or more sites of unsaturation. "Alkynyl" is intended
20 to include hydrocarbon chains of either a straight or branched configuration and one or more triple carbon-carbon bonds which may occur in any stable point along the chain, such as ethynyl and propynyl. These terms are not limited to monovalent radicals. The alkenyl or alkynyl groups may be unsubstituted or substituted. See
25 above for examples of substituents.

"Lower alkyl" means the above-defined broad definition of alkyl groups having 1 to 6 carbons in case of normal lower alkyl, and as applicable 3 to 6 carbons for lower branch chained (e.g., 2-methyl-propane) and cycloalkyl groups. "Lower alkenyl" is defined similarly having 2 to 6 carbons for normal lower alkenyl groups,
30 and 3 to 6 carbons for branch chained and cyclo- lower alkenyl groups. "Lower alkynyl" is also defined similarly, having 2 to 6 carbons for normal lower alkynyl groups, and 4 to 6 carbons for branch chained lower alkynyl groups. The lower alkyl,

alkenyl and alkynyl groups may be unsubstituted or substituted. See above for examples of substituents.

“Haloalkyl” is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen (for example $-C_vF_w$ where $v=1$ to 3 and $w=1$ to $(2v+1)$). The term haloalkyl is not limited to a monovalent radical. “Halogen” includes fluorine, chlorine, bromine, iodine and astatine. Examples of haloalkyl include, but are not limited to, trifluoromethyl, trichloromethyl, pentafluoroethyl, and pentachloroethyl.

10 The term “heterocyclic” refers to any compound in which the ring structure is a combination of more than one kind of atom. Examples of heterocyclic compounds include, but are not limited to, pyridine, furan and pyrrole. This term is not limited to a monovalent radical. The heterocyclic groups may be unsubstituted or substituted. See above for definition of substituents.

15 As used herein the term “prodrug” means a precursor or derivative form of a pharmaceutically active agent or substance that is less cytotoxic to a target cell as compared to the drug metabolite and is capable of being enzymatically activated or converted into the more active form.

A “composition” is intended to mean a combination of active agent and another compound or composition, inert (for example, a surface, a paint, a detectable agent or label or a pharmaceutically acceptable carrier) or active, such as an adjuvant or disinfectant.

A “pharmaceutical composition” is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

The term “prophylactically effective amount” refers to an amount effective in preventing infection in a subject or plant infestation.

The term “pharmaceutically acceptable carrier” and “biologically acceptable carrier” refer to a carrier or adjuvant that is administered to a host or patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is non-toxic, when administered in doses sufficient to deliver an effective amount of the compound. Examples of suitable carriers include liquid phase carriers, such as sterile or aqueous solutions, as well as those described below.

Examples of pharmaceutically acceptable carrier include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For
5 examples of carriers, stabilizers and adjuvants, see Martin, REMINGTON'S PHARM. SCI., 15th Ed. (Mack Publ. Co., Easton (1975)).

The term "treating" refers to any of the following: the alleviation of symptoms of a particular disorder in a patient; the improvement of an ascertainable measurement associated with a particular disorder; or a reduction in microbial number. One of skill
10 in the art can determine when a host has been "treated" by noting a reduction in microbial load or an alleviation in symptoms associated with infection.

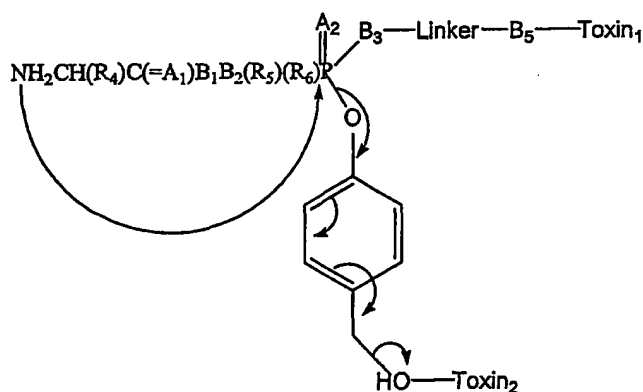
The term "pharmaceutically acceptable salt, prodrug or derivative" relates to any pharmaceutically acceptable salt, ester, ether, salt of an ester, solvate, such as ethanolate, or other derivative of a compound of the present invention which, upon
15 administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an active metabolite or residue thereof. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (*e.g.*, by allowing an orally administered compound to be more readily absorbed into
20 the blood) or which enhance delivery of the parent compound to a biological compartment (*e.g.*, the brain or lymphatic system).

Salts of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic,
25 salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid
30 addition salts. Examples of bases include alkali metal (*e.g.*, sodium) hydroxides, alkaline earth metal (*e.g.*, magnesium) hydroxides, ammonia, compounds of formula NW_4^+ , wherein W is C_{1-4} alkyl and THAM (2-amino-2-hydroxymethyl-1,3-propanediol).

Examples of salts include: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+ , Li^+ , NH_4^+ , and NW_4^+ (wherein W is a C_{1-4} alkyl group).

For therapeutic use, salts of the compounds of the present invention will be pharmaceutically acceptable. However, salts of acids and bases which are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound or for use to reduce microbial infestation in plants.

The term "traceless linker" indicates a spacer or connector between two parts of a single molecule such that when a particular bond is severed between the two parts of the molecule, the connector which is still attached to the second part of the molecule, eliminates leaving no trace of itself. This may most easily be seen by examining the following non-limiting example:



Once the PDF has deformedylated the prodrug to reveal the amine group, the amine nitrogen attacks the phosphorus to form a six-member ring with the resultant elimination of the oxygen-containing group, which in turn undergoes a semiquinone methide rearrangement resulting in the release of the toxin with no remaining trace of

the linker moiety itself, the linker thus being "traceless." Other traceless linkers will be apparent to those skilled in the art based on the disclosure herein and are within the scope of this invention. *See*, for example, de Groot et al. (2000).

The term "effective amount" is to include therapeutically or prophylactically effective amounts. The term refers to an amount effective in treating or preventing an infection in a patient or an infestation in a plant either as monotherapy or in combination with other agents.

"Inhibiting the growth" of a microorganism means reducing by contact with an agent, the rate of proliferation of such a microorganism, in comparison with a control microorganism of the same species not contacted with this agent.

A "subject" is any living entity, plant or animal, that is or can be a direct or indirect host to a PDF expressing microorganism. Thus, without limitation, crops such as corn, soybeans, grains, ornamental plants, trees, etc. are subjects within the scope of this invention as are animals such as, also without limitation, fish, shellfish, birds, mammals, and humans. Mammals include, but are not limited to, rodents, simians, farm animals, sport animals, pets and humans.

As used herein, "Toxin₁ and Toxin₂" are each an agent that is toxic upon activation by an activating enzyme, and may be the same or different. More generally, they each refer to any chemical entity useful to treat or prevent harm to plants or animals caused by pathogenic organisms that express PDF. Such chemical entities include, without limitation, anthelmintics, antibacterials including both antibiotics and synthetic compounds, antifungals, antineoplastics, antiprotozoal and the like. It is understood that the, in one aspect, either or both "Toxins" may include B₃, B₄, B₅ or B₆ depending on the structure of the prodrug and the mechanism of release of the active species from it. An example of this is seen in Fig. 1, wherein the oxygen attached to the "Tox" group is actually a component (as a hydroxyl group) of the actual Toxin, triclosan. Some specific examples of chemical entities that can comprise Toxin₁ and Toxin₂ are aminoglycosides, mitomycin, CC-1065, ducarmycin, cyclopropyl indole, cyclopropyl benzoindole analogs, anthracyclins, vinca alkaloids, mitomycins, bleomycins, penicillins, cephalosporins, oxacillins, carbopenems, tetracyclins, chloramphenicols, macrolides, cycloserines, fluoroquinolones (including, but not limited to, ciprofloxacin and norfloxacin), glycopeptides, aminoglycosides, peptide antibiotics, oxazolidinones, quinolones, sulfonamides, cytotoxic nucleosides,

pteridine family, nitrogen mustards, polyhalogenated biaryl ethers, diynes, podophillotoxins, taxoids, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloromethotrexate, mitomycin C, porfiromycin, 6-mercaptapurine, cytosine arabinoside, podophillotoxin, etoposide, etoposide phosphate, melphalan, vindesine, vinblastine, vincristine, leurosine, leurosine, bis- (2-chloroethyl)amine, trichlorcarban, trichlorocarbanilide, triclosan, tribromosalicylanilide, sulphamethoxazole, chloramphenicol, cycloserine, trimethoprim, chlorhexidine, hexachlorophene, fenticlor, 5-chloro-2-(2,4-dichlorophenoxy)phenol, 4-chloro-2-(2,4-dichlorophenoxy)phenol, 3-chloro-2-(2,4-dichlorophenoxy)phenol, 6-chloro-2-(2,4-dichlorophenoxy)phenol, 5-chloro-2-(3,4-dichlorophenoxy)phenol, 5-chloro-2-(2,5-dichlorophenoxy)phenol, 5-chloro-2-(3,5-dichlorophenoxy)phenol, 2,2'-dihydroxy biphenyl ether, halogenated 2-hydroxybenzophenones, 2-mercaptopyridine-N-oxide, combretastatin, camptothecin, apoptolidene, cisplatin, epothilone, halichondrin, hemicasterlin, methioprim, thapsigargin, chloroquine, 4-hydroxycyclophosphamide, etoposide, colchicine, melphalan, quercetin, genistein, erbstatin, N-(4-aminobutyl)-5-chloro-2-naphthalen-sulfonamide, pyridinyloxazol-2-one, isoquinolyloxazolone-2-one, verapamil, quinine, quinidine, and chloroquine.

PDF is a well-studied enzyme whose crystallographic structure has been elucidated. Chan et al. (1997). The enzyme has been expressed in *E. coli* BL21(DE3) cells Rajagopalan et al. (1997). The authors of the paper isolated the *E. coli* *def* gene by PCR using the primers designed based on the literature data on the sequence of the gene. The purified enzyme is unstable due to fast oxidation of the catalytic site Fe^{2+} by atmospheric oxygen. Rajagopalan et al. (1998). The conditions for proper handling of the enzyme to avoid inactivation have been reported. Rajagopalan et al. (1997). Zn^{2+} and Ni^{2+} containing PDF's are stable allowing for the *in vitro* evaluation of the enzyme catalytic properties. There exists a simple continuous colorimetric assay for PDF. Wei et al. (1997). It utilizes N-formylmethionylleucine p-nitroanilide as a substrate. A coupled aminopeptidase reaction that follows the PDF reaction releases p-nitroaniline that can be monitored spectrophotometrically at 405 nm.

PDF is a perfect ECTA target enzyme. It is active in bacteria and inactive in human hosts. It has broad substrate specificity. Deformylation liberates a free amino group of methionine (or another amino acid or an amino acid analog tolerated as an

R₃ of the substrate, such as norleucine) which can perform a subsequent nucleophilic attack. With a rationally designed dipeptide or an equivalent peptidomimetic fragment of the substrate the free amino group can attack an optimally positioned phosphorus of the substrate thus forming a cyclic molecule and releasing a toxin.

5 The substrate can be optimized to enhance cyclization. The scheme of the proposed reaction exemplifying a dipeptide-based substrate is given in Figure 1. Here X can be, for example, sulfur (methionine) or -CH₂- (norleucine). R₁ and R₂ are aliphatic radicals that can be selected based on the published SAR data for PDF. Hu et al. (1998).

10 This invention also provides a composition comprising the prodrug compounds as described above, alone or in combination with other compounds or other agents, known or yet to be discovered, and a carrier. In one aspect, the carrier is another molecule or an inert substance such as a plate or column. In an alternative embodiment, the carrier is a pharmaceutically acceptable carrier. Pharmaceutically
15 acceptable carriers are known in the art and described briefly above.

This invention also provides a composition comprising the prodrug compounds as described above, alone or in combination with other compounds or other agents, known or yet to be discovered, and a carrier. Any of the above noted compounds can be combined with a carrier, such as a pharmaceutically acceptable
20 carrier, and/or an additional effective agent such as an antibiotic. These compounds and compositions are also useful in combination with known or yet to be discovered therapeutics and therapeutic methods to enhance the therapy or efficacy of the drug.

Also provided by this invention is a method for inhibiting the growth of a PDF expressing microorganism by contacting the microorganism with an effective amount
25 of the compound as described above. Methods to detect PDF expression are known in the art. See, for example, Wei, et al. (1997). This method is particularly useful in inhibiting the growth of gram-positive and gram-negative microorganisms, e.g., *S. aureus*, *S. epidermidis*, *K. pneumoniae*, *E. aerogenes*, *E. cloacae*, *M. catarrhalis*, *E. coli*, *E. faecalis*, *H. influenzae* and *P. aeruginosa*. and those identified in Table 2,
30 below. This method can be practiced *in vitro*, *ex vivo* and *in vivo*. Further provided is a method for alleviating the symptoms of an infection in a subject, wherein the infection is caused by a PDF expressing microorganism, by administering or delivering to the subject an effective amount of the compound described above. Also

- provided by this invention is a method for treating an infection caused by a PDF expressing microorganism by administering or delivering to the subject an effective amount of the compound described above. A "subject" is defined above and includes mammals such as human patients. Examples of PDF expressing microorganisms and
- 5 the corresponding diseases and symptoms caused by infection by these microorganisms, are provided in Table 2, below.

Table 2

| <i>PDF Expressing Microorganism</i> | <i>Disease or Symptom Caused by Infection</i> |
|--|--|
| Gram-Positive | |
| <i>Staphylococcus aureus</i> | major human pathogen, bacteremia, pneumonia |
| <i>Staphylococcus epidermidis</i> and other coagulase-negative staphylococci | urinary tract infections, osteomyelitis, bacteremia |
| <i>Streptococcus pyogenes</i> | bacteremia, lymphagitis, pneumonia |
| <i>Streptococcus pneumoniae</i> | pneumonia, otitis media, sinusitis |
| <i>Streptococcus agalactiae</i> | primary bacteremia, pneumonia, endocarditis, osteomyelitis |
| <i>Enterococcus species</i> | urinary tract infections, bacteremia, endocarditis, intra-abdominal and pelvic infections, neonatal sepsis |
| | |

| <i>PDF Expressing Microorganism</i> | <i>Disease or Symptom Caused by Infection</i> |
|---|---|
| Gram-Negative | |
| <i>Neisseria gonorrhoeae</i> | genital infection, perihepatitis |
| <i>Moraxella catarrhalis</i> | otitis media, lower respiratory tract infections, pneumonia, bacteremia |
| <i>Campylobacter jejuni</i> | acute enteritis, acute colitis, bacteremia |
| <i>Enterobacteriaceae</i> (including <i>Escherichia</i> , <i>Salmonella</i> , <i>Klebsiella</i> , <i>Enterobacter</i>) | enteric infections, urinary tract infections, respiratory infections, bacteremia |
| <i>Pseudomonas aeruginosa</i> | endocarditis, respiratory infections, bacteremia, central nervous system infections |
| <i>Acinetobacter species</i> | respiratory tract infections, bacteremia, genitourinary |
| <i>Haemophilus influenzae</i> | meningitis, epiglottitis, pneumonia, bacteremia |

In the clinical use of the prodrug, antibiotics will likely follow well established guidelines. Dosage will likely be similar to those already employed for most other antibiotics. It is estimated that a dose of prodrug will be in the range of 100 µg to 100 mg to 1 gm, or given once every eight hours, or once a day, for one or two weeks, or until the patient tests negative for infectious organisms.

The compounds of this invention are expected to be administered in doses that reflect the literature doses for the active compounds of which they are prodrugs, with the difference in molecular weight being taken into account. Thus, it is estimated that the dosage for a prodrug hereof will typically be in the range of 100 µg to 100 mg to 1 gm.

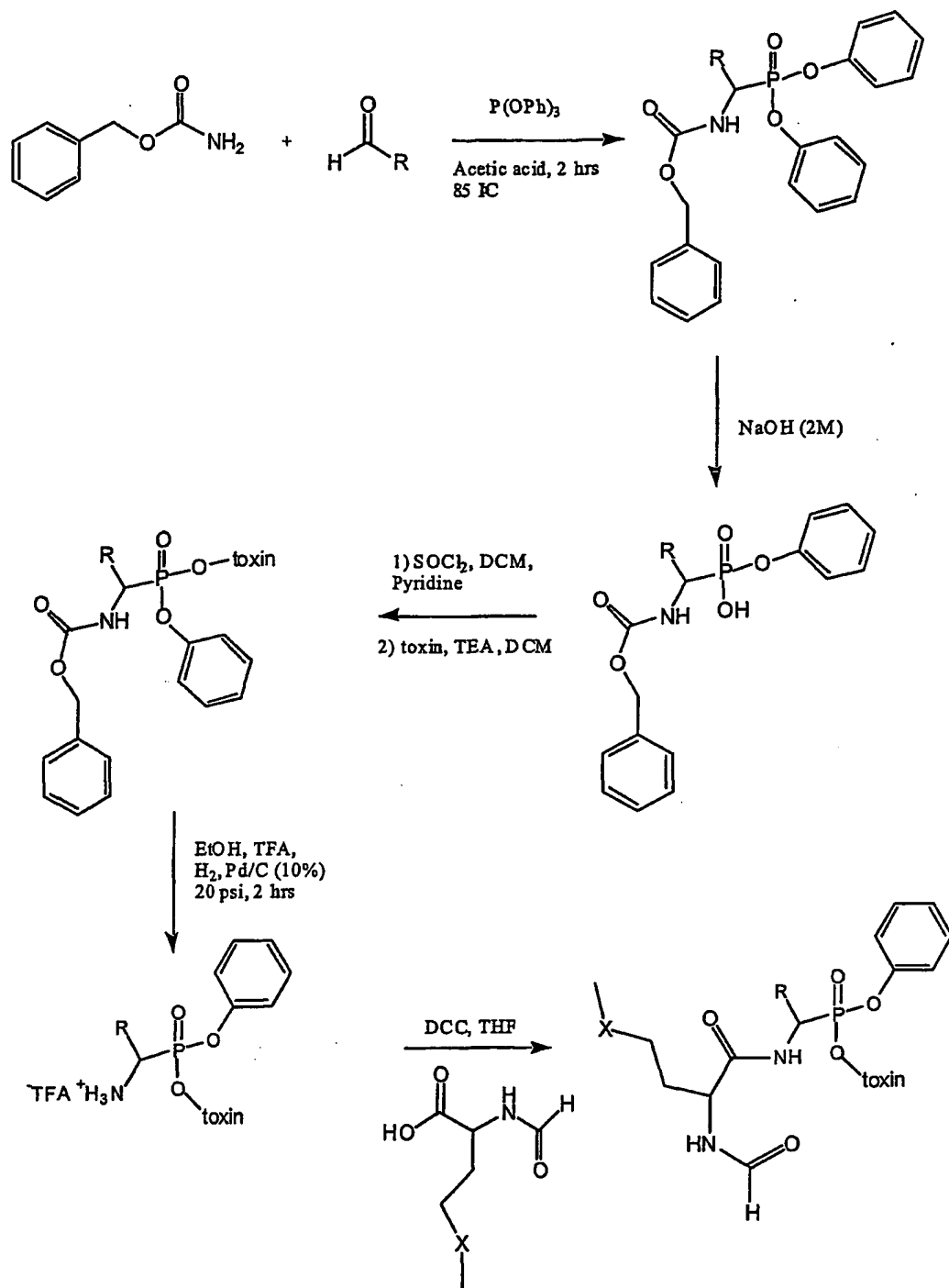
In one aspect, the invention encompasses a method of treating or protecting plants from infections caused by PDF expressing microorganisms by applying an effective amount of the substrate prodrug.

5 In order to achieve good dispersion and adhesion of the compounds to treat plants, it may be advantageous to formulate the compounds with components that aid dispersion and adhesion. Suitable formulations will be known to those skilled in the art.

10 This invention also provides a method for treating or protecting plants from infection by microorganisms expressing PDF by applying an effective amount of the prodrug compound to the foliage, roots or the soil surrounding the plants or roots. These isolated compounds can be combined with known pesticides or insecticides.

15 Compounds within the present invention when used to treat or protect plants from infections caused by PDF expressing microorganisms can be formulated as wettable powders, granules and the like, or can be microencapsulated in a suitable medium and the like. Examples of other formulations include, but are not limited to soluble powders, wettable granules, dry flowables, aqueous flowables, wettable dispersible granules, emulsifiable concentrates and aqueous suspensions. Other suitable formulations will be known to those skilled in the art.

20 This invention further provides a method for administering the prodrug compound to fish in an amount effective to either prevent or treat an infection caused by PDF expressing microorganisms. The compound may be administered by incorporating the compound into the food supply for the fish. Alternatively, the compound may be added to the water in which fish live, or are contained within. Finally, the compound may be administered to the fish as a suitable pharmaceutical
25 preparation. Other suitable formulations will be known to those skilled in the art. Further provided is a process for producing the prodrugs of this invention. In general the process requires the following steps:



With respect to the above diagram, X can be sulfur (methionine) or $\text{-CH}_2\text{-}$ (norleucine). R can be R_5 or R_6 as defined above. Reaction conditions and full names for the abbreviations can be found in the experimental examples *infra*.

- 5 This invention provides a method for identifying potential therapeutic agents that inhibit the growth of an organism expressing PDF by contacting a sample

containing the PDF expressing microorganism with an effective amount of a candidate prodrug compound. In a separate sample, the same microorganism is contacted with an effective amount of a prodrug of this invention. If the agent has comparable anti-proliferative ability as compared to a prodrug as described herein, the
5 candidate is useful to inhibit the growth of or kill a PDF-expressing microorganism.

The prodrug is contacted with the sample under conditions that favor the activation of the prodrug by PDF and then the sample is assayed for growth inhibition or microbial death. Alternatively, the sample can be tested for the presence of the byproducts of the reaction of PDF on the substrate. Varying amounts of the substrate
10 are contacted with a microorganism that expresses PDF for an amount of time effective for PDF to release the toxin from the cell, the bacteria is lysed and the analytes are analyzed using methods known in the art (*e.g.*, High Performance Liquid Chromatography ("HPLC")) to identify the reaction products.

Varying concentrations of the potential agent are contacted with the sample to
15 determine the optimal effective concentration of the agent. Thus, in one aspect, this invention relates to the discovery, and use thereof, of agents that are selective substrates for PDF.

Also provided by this invention are kits containing the prodrugs as described herein and instructions necessary to perform the screen.

20 The methods of the invention can be practiced *in vitro*, *ex vivo* or *in vivo*. *In vivo* practice of the invention in an animal such as a rat or mouse provides a convenient animal model system that can be used prior to clinical testing of the therapeutic agent or prodrug. In this system, a potential prodrug will be successful if microbial load is reduced or the symptoms of the infection are ameliorated compared
25 to an untreated, infected animal. It also can be useful to have a separate negative control group of cells or animals which has not been infected, which provides a basis for comparison.

When practiced *in vivo*, the candidate prodrug is administered or delivered to the animal in effective amounts. As used herein, the term "administering" for *in vivo*
30 and *ex vivo* purposes means providing the subject with an effective amount of the candidate prodrug effective to reduce microbial load. In these instances, the agent or prodrug may be administered with a pharmaceutically acceptable carrier. The agents, prodrugs and compositions of the present invention can be used in the manufacture of

medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

5 Methods of administering pharmaceutical compositions are known to those of ordinary skill in the art and include, but are not limited to, microinjection, intravenous or parenteral administration. The compositions are intended for topical, oral, or local as well as intravenous, subcutaneous and intramuscular administration.

Administration can be effected continuously or intermittently throughout the course of the treatment. Methods of determining the most effective means and dosage of

10 administration are known to those of skill in the art and will vary with the prodrug used for therapy, the purpose of the therapy, the microorganism being treated, the severity of the infection, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. For example, the compositions can be administered to a

15 subject already suffering from an antibiotic resistant bacterial infection. In this situation, an effective "therapeutic amount" of the composition is administered to prevent continued and to at least partially arrest microbial growth and proliferation and ameliorate the symptoms associated with an infection.

However, the prodrugs can be administered to subjects or individuals

20 susceptible to or at risk of developing an infection. In these embodiments, a "prophylactically effective amount" of the composition is administered to maintain cell viability and function at a level near to the pre-infection level.

Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most

25 effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can

30 be found below.

The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also take

the form of suspensions, solutions and emulsions of the active ingredient in aqueous or nonaqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds of the invention.

5 More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including oral, rectal, nasal, topical (including transdermal, aerosol, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It will also be appreciated that the route will vary with
10 the condition and age of the recipient, and the disease being treated.

 Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous injection of the agent, optionally in saline, or orally administered, for example, as a tablet, capsule or syrup containing the active ingredient. Desirable
15 blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue. The use of operative combinations is contemplated to provide therapeutic combinations requiring a lower total dosage of each component agent than may be required when each individual therapeutic compound or drug is used alone, thereby reducing adverse effects.

20 While it is possible for the agent to be administered alone, it is typical to present it as a pharmaceutical formulation comprising at least one active ingredient, as defined above, together with one or more pharmaceutically acceptable carriers therefor and optionally other therapeutic agents. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not
25 injurious to the patient.

 Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary administration. The formulations may conveniently be presented in unit dosage form and may be prepared
30 by any methods known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier that constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly

and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution
5 or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or
10 more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (*e.g.*, povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (*e.g.*, sodium starch glycolate, cross-linked povidone, cross-linked sodium
15 carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying
20 proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin
25 and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical compositions for topical administration according to the present invention may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol or oil. Alternatively, a formulation may
30 comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active ingredients and optionally one or more excipients or diluents.

If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, *i.e.*, an alcohol having two or more

hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound that enhances absorption or penetration of the agent through the skin or other affected areas. Examples of such dermal penetration
5 enhancers include dimethylsulfoxide and related analogues.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. A hydrophilic
10 emulsifier can be included together with a lipophilic emulsifier that acts as a stabilizer. In one variation, it includes both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

15 Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils
20 likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream can be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl
25 palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye
30 drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the agent.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the agent, such carriers as are known in the art to be appropriate.

Formulations suitable for nasal administration, wherein the carrier is a solid,
5 include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered as a dry powder or in an inhaler device by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer,
10 include aqueous or oily solutions of the agent.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may
15 include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile
20 liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Unit dosage formulations of interest include those containing a daily dose or unit, daily subdose, as herein above-recited, or an appropriate fraction thereof, of a
25 agent.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as
30 sweeteners, thickeners and flavoring agents. It also is intended that the agents, compositions and methods of this invention be combined with other suitable compositions and therapies.

These agents of this invention and the above noted compounds and their derivatives may be used for the preparation of medicaments for use in the methods described herein.

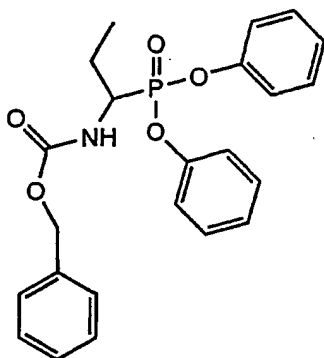
The following examples are intended to illustrate, but not limit the invention.

5 **Example 1 – Synthetic Scheme**

The following examples of the synthesis of the claimed compounds are meant to be illustrative of the synthesis of the invention and not limit the synthesis of the invention. The synthetic methods, as for example seen below, used to synthesize the compounds are known to those skilled in the art of organic and medicinal chemistry.

10 Substitutions, modifications or additional steps used in the synthesis of the compounds of the present invention known to those skilled in the art of organic and medicinal chemistry are anticipated. *See, e.g.,* J. March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS AND STRUCTURE, 4th edition (John Wiley & Sons, NY (1992)). In addition, it is understood that, for compounds having one or
15 more asymmetric centers, both the racemic mixture and enantiomerically enhanced (i.e., one enantiomer exists in greater than 50% of the mixture) or enantiomerically pure (one enantiomer comprises 90% or more of the mixture) compounds are within the scope of this invention.

20 **Synthesis of compound 1**

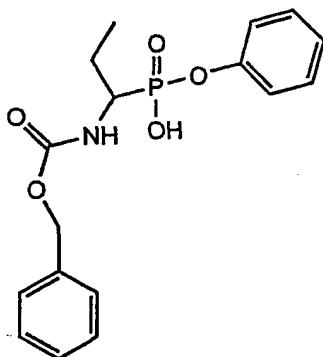


The synthesis of the 1-(N-benzyloxycarbonyl)-aminopropylphosphonate diphenyl ester has been performed following the method of Oleksyszyn (Synthesis, 1979, pp 985-986). A mixture of triphenyl phosphite (27.1 mL, 100 mmol), propanale
25 (11.25 mL, 150 mmol), benzyl carbamate (15.28 g, 100 mmol) and glacial acetic acid

(15 mL) was stirred for 1 hour at room temperature. The mixture was then heated at 85 °C for 1 hour. The volatile products were removed on a rotary evaporator on a boiling water bath obtaining an oily residue that was purified by column chromatography (silica gel; eluent: 40% ethyl acetate in hexane).

5 ³¹P NMR (CDCl₃, 500 MHz): 18.15; ¹H NMR (CDCl₃, 500 MHz): 1.09 (t, 3H), 1.79 (m, 1H), 2.11 (m, 1H), 4.42 (m, 1H), 5.12 (s, 2H), 7.0-7.38 (m, 15H).

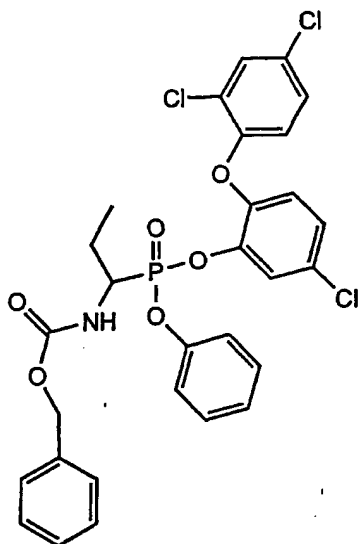
Synthesis of compound 2



10

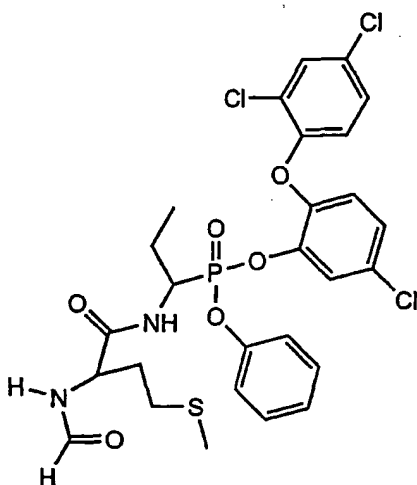
Compound 1 (4.0 g, 9.4 mmol) was partially dissolved in 2M aqueous solution of sodium hydroxide (20 mL). The resulting suspension was stirred at room temperature for 18 hours. The reaction mixture was then washed with dichloromethane (2 X 40 mL) and the aqueous layer was acidified (pH 0) with 2N
15 sulfuric acid (45 mL). The aqueous layer was extracted with dichloromethane (85 mL) and then with ethyl acetate (2 X 85 mL). The organic phase was dried over sodium sulfate for 30 minutes. After filtration of the drying agent and evaporation of the volatiles, compound 2 was obtained (2.98 g, 8.5 mmol); ³¹P NMR (D₂O, 500 MHz): 19.21; ¹H NMR (D₂O, 500 MHz): 0.94 (t, 3H, J = Hz), 1.58 (m, 1H), 1.90 (m, 1H), 3.76 (m, 1H), 5.0 (s, 2H), 7.06 (m, 2H), 7.15 (m, 1H), 7.36 (m, 2H), 7.41 (m, 4H).
20

Synthesis of compound 3



Compound 2 (1.3 g, 3.72 mmol), was partially dissolved in anhydrous
 5 dichloromethane (10 mL). Thionyl chloride (800 μ L) was added to the mixture
 followed by addition of a catalytic amount of anhydrous pyridine (10 μ L). The
 resulting reaction mixture was stirred under argon atmosphere for 30 minutes. The
 volatiles were removed under *vacuo*, and the residue was left under high *vacuo* for 2
 hours. The crude phosphonochloridate was then redissolved in anhydrous
 10 dichloromethane (5 mL) and the mixture was added dropwise, at 0 $^{\circ}$ C, to a stirring
 solution of triclosan (1.07 g, 1 eq.) and anhydrous triethylamine (520 μ L, 1 eq.) in
 anhydrous dichloromethane (5 mL). The reaction mixture was allowed to warm up to
 room temperature and was stirred under argon atmosphere for 1 hour. The reaction
 mixture was then extracted with water (10 mL). The organic phase was dried over
 15 sodium sulfate for 30 minutes. After filtration of the drying agent and evaporation of
 the volatiles by vacuum distillation, the residue was purified by column
 chromatography (silica gel; eluent: 20% ethyl acetate in hexane) obtaining compound
 3 (550 mg); ^{31}P NMR (CDCl_3 , 500 MHz): 19.08, 19.29; ^1H NMR (CDCl_3 , 500 MHz):
 1.03 (m, 3H), 1.67 (m, 1H), 2.05 (m, 1H), 4.41 (m, 1H), 5.08 (m, 2H), 6.73 (m,
 20 1H), 6.83(m, 2H), 7.13 (m, 5H), 7.30 (m, 8H).

Synthesis of compound 4



Compound 3 (200 mg, 0.322 mmol) was dissolved in absolute ethanol (80 mL) and then added with Pd 10% on carbon (80 mg) and trifluoroacetic acid (40 ul). The mixture was then shaken under hydrogen atmosphere (10 psi) for 30 minutes. After filtration through a pad of celite, the solution was concentrated to dryness, and the resulting dense oil was left under high *vacuo* for 2 hours. The oil was then dissolved in anhydrous tetrahydrofuran (5 mL), and the resulting solution was added to a stirring solution of N-formyl methionine (60 mg) and 1,3-dicyclohexyl carbodiimide (66 mg) in anhydrous tetrahydrofuran (3 mL). Anhydrous triethylamine (50 ul,) was then added to the mixture, and the reaction was allowed to stir for 12 hours under argon atmosphere at room temperature. After the filtration of the precipitated material, the mixture was extracted with water (10 mL). The organic phase was dried over sodium sulfate for 30 minutes. After filtration of the drying agent and evaporation of the volatiles by vacuum distillation, the residue was purified by preparative HPLC (reverse phase, C18; gradient from 50% acetonitrile in water to 100% acetonitrile over 40 minutes). The desired compound was isolated as a mixture of diastereoisomers with retention time of 12.28 minutes. ³¹P NMR (CDCl₃, 500 MHz): 18.04, 18.16, 18.32; ¹H NMR (CDCl₃, 500 MHz) selected signals: 1.25 (m, 5H), 1.07-2.09 (m, 7H), 4.65 (m, 1H), 6.7-7.47 (m, 11H), 8.12 (s, 1H); m/e 645.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the invention. Other aspects,

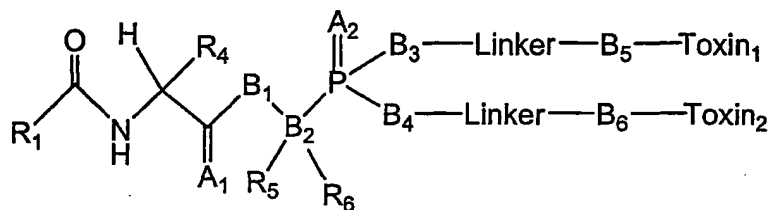
advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

References

- Apfel, C. et al. (2000) *J. Med. Chem.* 43:2324-2331.
- Apfel et al. (2001a) *Anti. Agents and Chemo.* 45(4):1053-1057.
- 5 Apfel et al. (2001b) *Anti. Agents and Chemo.* 45(4):1058-1064.
- Becker, A. et al. (1998) *Nat. Struct. Biol.* 5(12): 1053-8.
- Chan, M.K. et al. (1997) *Biochemistry* 36(45):13904-9.
- Chen, D.Z. et al. (2000) *Biochemistry* 39(6):1256-62.
- Clements, J.M. et al. (2001) *Anti. Agents and Chemo.* 45(2):563-570.
- 10 de Groot, F.M.H. et al. (2000) *J. Med. Chem.* 43:3093-3102.
- Durand et al. (1999) *Arch. Bio. And Biophysics.* 367(2): 297-302.
- Giglione, C. et al. (2000a) *Mol. Microbiol.* 36:1197205.
- Giglione, C. et al. (2000b) *EMBO J.* 19(21):5916-5929.
- Guilloteau J-P. et al. (2002) *J. Mol. Biol.* 320(5):951-962
- 15 Hao, B. et al. (1999) *Biochemistry* 38(15):4712-9.
- Hu, Y.J. et al. (1998) *Bioorg Med Chem Lett.* 8(18):2479-82.
- Huntington, K.M. et al. (2000) *Biochemistry* 39(15):4543-51.
- Jayasekera, M.M. et al. (2000) *Arch. Biochem. Biophys.* 381(2):313-316.
- Meinzel, T. (1999) *Pathol. Biol.* 47:780-783.
- 20 Meinzel, T. et al. (1993) *Biochimie.* 75(12):1061-75. Review.
- Nelson, D.L. et al. (2000) *Principles of Biochemistry.* 2000, ed. Lehninger,
- Ragusa S., et al. (2000) *J.Mol.Biol.*, 280, 551-523.
- Rajagopalan, P.T. et al. (1997) *Biochemistry* 36(45):13910-8.
- Rajagopalan, P.T. et al. (1998) *Biol Chem.* 273(35):22305-10.
- 25 Wei Y. et al. (1997) *Anal Biochem.* 250(1):29-34.
- Wei, Y. et al. (2000) *J Comb Chem.* 2(6):650-7.
- Wei, Y. et al. (2000b) *Bioorg Med Chem Lett.* 10(10):1073-6.
- Wise, R. et al (2002) *Anti. Agents and Chemo*, 46(4):1117-1118
- Yuan, Z. et al. (2001) *Drug Discovery Today*, 6(18): 954-961

WHAT IS CLAIMED:

1. A compound having the structure:



5

wherein:

R_1 is hydrogen or CH_3 ;

- 10 R₄ is selected from the group consisting of a substituted or unsubstituted aryl group, a substituted or unsubstituted, saturated or unsaturated C₁ – C₆ alkyl group and –CH₂CH₂XCH₃, wherein X is selected from the group consisting of oxygen, sulfur and CH₂;

- 15 R₅ and R₆ are independently selected from the group consisting of hydrogen, a substituted or unsubstituted C₅–C₁₄ aryl group and a substituted or unsubstituted, saturated or unsaturated C₁–C₆ alkyl group;

=A₁ is either absent, in which case the carbon to which it was attached becomes a CH₂

- 20 group, or A₁ is selected from the group consisting of oxygen, sulfur, NH, NOH, NR₈, and CR₉R₁₀, wherein R₈, R₉ and R₁₀ are independently selected from the group consisting of hydrogen and C₁ – C₆ alkyl;

=A₂ is either absent, in which case the carbon to which it was attached becomes a CH₂

- 25 group, or A₂ is selected from the group consisting of oxygen, sulfur, NH, NOH and NR₁₁, wherein R₁₁ is selected from the group consisting of hydrogen and C₁ - C₅ alkyl;

B₁ is either absent or is selected from the group consisting of oxygen, sulfur, NR₁₂ and

CR₁₃R₁₄, wherein R₁₂, R₁₃ and R₁₄ are independently selected from the group consisting of hydrogen and a substituted or unsubstituted saturated or unsaturated alkyl;

- 5 B₂ is selected from the group consisting of carbon, oxygen and nitrogen, wherein, when B₂ is oxygen, R₅ and R₆ are absent and when B₂ is nitrogen, one of R₅ or R₆ is absent;

- B₁ may be joined through R₁₂, R₁₃ or R₁₄ to R₅ or R₆ to form a saturated or
10 unsaturated, substituted or unsubstituted ring which may contain 0 – 3 nitrogen atoms and/or 0 – 1 oxygen or sulfur atoms;

- B₃ and B₄ are independently absent or selected from the group consisting of oxygen, sulfur, NH, CH₂, NR₁₇, CHR₁₈, CR₂₁R₂₂ and, when either linker₁-B₅-Toxin₁ or linker₂-
15 B₆-Toxin₂ is absent, OR₁₅, SR₁₆, NR₁₉R₂₀ and CR₂₃R₂₄R₂₅, wherein R₁₅ – R₂₅ are independently selected from the group consisting of hydrogen, substituted or unsubstituted, saturated or unsaturated alkyl and substituted or unsubstituted aryl;

- B₃ or B₄ may be joined through one of R₁₅ - R₂₅ to B₁, R₅ or R₆ to form a saturated or
20 unsaturated, substituted or unsubstituted ring which contains one phosphorus atom and which may contain 0 – 3 nitrogen atoms and/or 0 – 1 oxygen or sulfur atoms;

- B₅ and B₆ are independently absent or selected from the group consisting of oxygen, sulfur, OC(=O), SC(=S), OC(=O)NH, SC(=S)NH, OC(=S)NH, N(R₂₆) and
25 C(R₂₇)(R₂₈), wherein R₂₆, R₂₇ and R₂₈ are independently selected from the group consisting of hydrogen and a substituted or unsubstituted, saturated or unsaturated alkyl;

- Linker₁ and Linker₂ are independently absent or present and if present are traceless;
30 and,

one of Toxin₁ or Toxin₂ may be absent.

2. The compound of claim 1, wherein:

R₁ is hydrogen; and,

R₄ is CH₂CH₂XCH₃, wherein X is selected from the group consisting of CH₂, sulfur
5 and oxygen.

3. The compound of claim 2, wherein X is sulfur.

4. The compound of claim 3, wherein A₁ and A₂ are both oxygen.

10

5. The compound of claim 4, wherein Linker₁-B₅-Toxin₁ is absent.

6. The compound of claim 5, wherein B₁ is absent.

15

7. The compound of claim 5, wherein B₂ is carbon.

8. The compound of claim 5, wherein B₂ is nitrogen.

9. The compound of claim 5, wherein B₃ is selected from the group
20 consisting of OH, OCH₃, OCH₂CH₃ and OC₆H₅.

10. The compound of claim 5, wherein Linker₂ is selected from the group
consisting of

substituted or unsubstituted allyl;

25

substituted or unsubstituted benzyl,

C₆H₄CH₂X₁C(=X₂), wherein X₁ and X₂ are independently selected from the
group consisting of oxygen, sulfur and N(R₂₉), wherein R₂₉ is hydrogen or C₁
– C₆ alkyl; and,

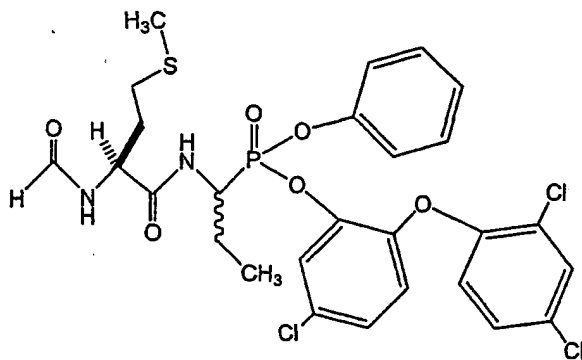
(CH₂)_nN(R)C(=O), wherein n is 2 or 3 and R is hydrogen or C₁ – C₆ alkyl.

30

11. The compound of claim 10, wherein B₆ is absent.

12. The compound of claim 1, wherein Toxin₁ and Toxin₂ are independently selected from the group consisting of aminoglycosides, mitomycin, CC-1065, ducarmycin, cyclopropyl indole, cyclopropyl benzoindole analogs, anthracyclins, vinca alkaloids, mitomycins, bleomycins, penicillins, cephalosporins, oxacillins, carbopenems, tetracyclins, chloramphenicols, macrolides, cycloserines, fluoroquinolones (including, but not limited to, ciprofloxacin and norfloxacin), glycopeptides, aminoglycosides, peptide antibiotics, oxazolidinones, quinolones, sulfonamides, cytotoxic nucleosides, pteridine family, nitrogen mustards, polyhalogenated biaryl ethers, diynes, podophillotoxins, taxoids, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloromethotrexate, mitomycin C, porfiromycin, 6-mercaptopurine, cytosine arabinoside, podophillotoxin, etoposide, etoposide phosphate, melphalan, vindesine, vinblastine, vincristine, leurosine, leurosine, bis-(2-chloroethyl)amine, trichlorcarban, trichlorocarbanilide, triclosan, tribromosalicylanilide, sulphamethoxazole, chloramphenicol, cycloserine, trimethoprim, chlorhexidine, hexachlorophene, fentichlor, 5-chloro-2-(2,4-dichlorophenoxy)phenol, 4-chloro-2-(2,4-dichlorophenoxy)phenol, 3-chloro-2-(2,4-dichlorophenoxy)phenol, 6-chloro-2-(2,4-dichlorophenoxy)phenol, 5-chloro-2-(3,4-dichlorophenoxy)phenol, 5-chloro-2-(2,5-dichlorophenoxy)phenol, 5-chloro-2-(3,5-dichlorophenoxy)phenol, 2,2'-dihydroxy biphenyl ether, halogenated 2-hydroxybenzophenones, 2-mercaptopyridine-N-oxide, combretastatin, camptothecin, apoptolidene, cisplatin, epothilone, halichondrin, hemiasterlin, methioprim, thapsigargin, chloroquine, 4-hydroxycyclophosphamide, etoposide, colchicine, melphalan, quercetin, genistein, erbstatin, N-(4-aminobutyl)-5-chloro-2-naphtalen-sulfonamide, pyridinyloxazol-2-one, isoquinolyloxazolone-2-one, verapamil, quinine, quinidine, and chloroquine.
13. The compound of claim 11, wherein Toxin₂ is selected from the group consisting of aminoglycosides, mitomycin, CC-1065, ducarmycin, cyclopropyl indole, cyclopropyl benzoindole analogs, anthracyclins, vinca alkaloids, mitomycins, bleomycins, penicillins, cephalosporins, oxacillins, carbopenems, tetracyclins, chloramphenicols, macrolides, cycloserines, fluoroquinolones (including, but not limited to, ciprofloxacin and norfloxacin), glycopeptides, aminoglycosides, peptide antibiotics, oxazolidinones, quinolones, sulfonamides, cytotoxic nucleosides,

- pteridine family, nitrogen mustards, polyhalogenated biaryl ethers, diyne-
nes, podophyllotoxins, taxoids, doxorubicin, carminomycin, daunorubicin, aminopterin,
methotrexate, methopterin, dichloromethotrexate, mitomycin C, porfiromycin, 6-
mercaptapurine, cytosine arabinoside, podophyllotoxin, etoposide, etoposide
5 phosphate, melphalan, vindesine, vinblastine, vincristine, leurosine, leurosine, bis-
(2-chloroethyl)amine, trichlorcarban, trichlorocarbanilide, triclosan,
tribromosalicylanilide, sulphamethoxazole, chloramphenicol, cycloserine,
trimethoprim, chlorhexidine, hexachlorophene, fentichlor, 5-chloro-2-(2,4-
dichlorophenoxy)phenol, 4-chloro-2-(2,4-dichlorophenoxy)phenol, 3-chloro-2-(2,4-
10 dichlorophenoxy)phenol, 6-chloro-2-(2,4-dichlorophenoxy)phenol, 5-chloro-2-(3,4-
dichlorophenoxy)phenol, 5-chloro-2-(2,5-dichlorophenoxy)phenol, 5-chloro-2-(3,5-
dichlorophenoxy)phenol, 2,2'-dihydroxy biphenyl ether, halogenated 2-
hydroxybenzophenones, 2-mercaptopyridine-N-oxide, combretastatin, camptothecin,
apoptolidene, cisplatin, epothilone, halichondrin, hemicasterlin, methioprim,
15 thapsigargin, chloroquine, 4-hydroxycyclophosphamide, etoposide, colchicine,
melphalan, quercetin, genistein, erbstatin, N-(4-aminobutyl)-5-chloro-2-naphthalen-
sulfonamide, pyridinyloxazol-2-one, isoquinolyloxazolone-2-one, verapamil, quinine,
quinidine, and chloroquine.
- 20 14. The compound of claim 13, wherein Toxin₂ is a quinolone.
15. The compound of claim 13, wherein Toxin₂ is selected from the group
consisting of triclosan, cyclopropylindole, cyclopropylbenzoindole and derivatives
thereof.
- 25 16. The compound of claim 5, wherein B₁ is joined to R₅ or R₆ to form a ring.
17. A compound of claim 1, having the structure:



18. A composition comprising:

5 a compound of claim 1; and,

a pharmaceutically acceptable carrier.

19. A method for the treatment of a disease caused by a microorganism expressing
10 a peptide deformylase enzyme, comprising administering an effective amount of a
compound of claim 1 to a patient in need thereof.

o

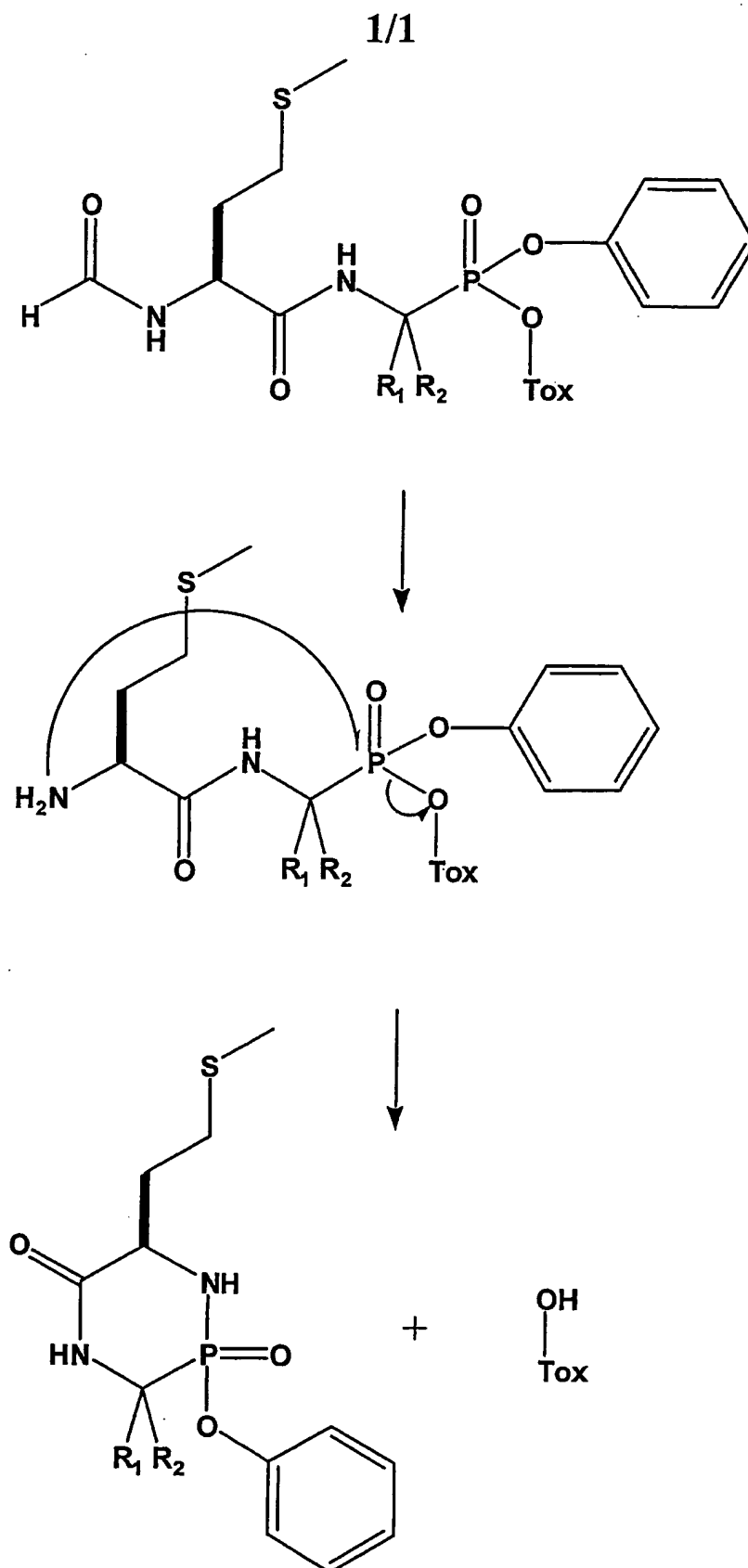


Figure 1

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.